CASE REPORT

Pregnancy and delivery after cryopreservation of zygotes produced by in-vitro matured oocytes retrieved from a woman with polycystic ovarian syndrome

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This case report describes the birth of a healthy infant after cryopreservation of zygotes produced by in-vitro matured oocytes retrieved from an anovulatory woman with polycystic ovarian syndrome (PCOS). To initiate the treatment cycle, the patient received intravaginal progesterone at night for 10 days to induce a withdrawal bleed. Oocyte retrieval was performed on day 11 following a withdrawal bleed. The patient was administered 10 000 IU of HCG subcutaneously 36 h prior to oocyte collection. A total of 63 immature oocytes were obtained; 10 were morphologically abnormal. Following incubation for 24–48 h in the maturation medium, TC-199 supplemented with 20% patient’s own serum, 75 mIU/ml FSH and LH, 77.4% (41/53) of the oocytes were at the metaphase-II stage. Thirty-one (31/41, 75.6%) were fertilized using ICSI with her husband’s spermatozoa, 15 fertilized oocytes were cultured for embryo transfer and 16 were frozen at the pronuclear stage. Pregnancy ensued following fresh embryo transfer. Unfortunately, the pregnancy was miscarried eight weeks later. However, the second frozen–thawed embryo transfer attempt resulted in a full-term pregnancy with delivery of a healthy male infant.

Key words: Cryopreservation/immature oocytes/ICSI/pregnancy/PCOS

Introduction

Women with polycystic ovarian syndrome (PCOS) are characterized by abnormal endocrine parameters, anovulation, numerous antral follicles within their ovaries and frequently infertility (Goldzieher and Green, 1962). Patients with PCOS are extremely sensitive to stimulation with exogenous gonadotropin and are at increased risk of developing ovarian hyperstimulation syndrome (OHSS) when treated with gonadotrophins for assisted reproduction (MacDougall et al., 1993). Recovery of immature oocytes followed by in-vitro maturation (IVM) of these oocytes could be developed as a new method for the treatment of patients with infertility due to PCOS. Methods developed by Trounson for transvaginal ultrasound guided recovery of immature oocytes from the ovaries of patients with PCOS introduced IVM firmly into the clinical realm (Trounson et al., 1994). It has been noted that most of the follicles from patients with PCOS are not atretic and these oocytes appear to have developmental competence (Almahbobi et al., 1996; Barnes et al., 1996). However, the maturation rate of immature oocytes retrieved from women with PCOS is lower than that of those retrieved from women with normal menstrual cycles (Cha and Chian, 1998). It has been reported that priming with human chorionic gonadotrophin (HCG) before immature oocyte retrieval in PCOS patients improves oocyte maturation, fertilization, embryo development and pregnancy rates (Chian et al., 1999, 2000). However, it is still unclear whether the embryos produced by in-vitro matured oocytes can be frozen for further embryo transfer. In this case report, we present the live birth of a healthy infant resulting from transfer of frozen-thawed embryos produced from in-vitro matured oocytes, which were cryopreserved at the pronuclear stage following intracytoplasmic sperm injection (ICSI). To the best of our knowledge, this is the first report of the birth of an infant from cryopreserved embryos (zygotes) produced by in-vitro matured oocytes derived from an unstimulated patient with PCOS.

Case report

A 31-year-old woman with PCOS presented with irregular menstrual cycles, anovulation and 4 years of infertility. She had failed to achieve pregnancy following 6 cycles of intrauterine insemination (IUI) with her husband’s spermatozoa combined with ovulation induction using clomiphene citrate or gonadotrophin therapy. The patient had hirsutism, a baseline LH/FSH ratio of 2.76, and enlarged polycystic ovaries which met the
ultrasound diagnostic criteria of PCOS (Adams et al., 1986). Routine semen analysis on her husband indicated no abnormality. To initiate the treatment cycle the patient received intravaginal progesterone (Prometrium; Schering, Pointe-Clair, Quebec, Canada) in a dose of 300 mg a night for 10 days to induce a withdrawal bleed. After stopping the progesterone treatment, menstrual bleeding started 3 days later. She had a baseline ultrasound scan to ensure no ovarian cysts were present and transvaginal ultrasound scans were performed on day 6 and day 8 to exclude the development of a dominant follicle. Oocyte retrieval was performed on day 11. The patient was administered 10 000 IU of HCG (Profasi; Serono, Oakville, ON, Canada) s.c. 36 h prior to immature oocyte collection. Transvaginal ultrasound guided oocyte collection was done using a specially designed aspiration needle (Cook, Eight Mile Plains, Queensland, Australia, K-OPS-1235-Wood) with an aspiration pressure of 7.5 kPa. Aspiration of all small follicles (2–8 mm diameter) was attempted under spinal anesthesia. In total more than 70 follicles were aspirated from both ovaries. Aspirates were collected in 10 ml culture tube (Falcon; Franklin Lakes, NJ, USA) containing 2 ml warm 0.9% saline with 2 IU/ml heparin (Baxter, Toronto, Canada). Following oocyte collection, the maturity of the oocytes was determined under microscopy and the immature oocytes were then transferred to maturation medium for culture. All oocyte handling procedures were conducted on warm stages and plates at 37°C. Cumulus oocyte complexes were washed in TC-199 medium (Sigma, St Louis, MO, USA) with 10% patient’s own serum (inactivated at 56°C for 30 min) and the immature oocytes were then incubated in the maturation medium, TC-199 medium supplemented with 20% patient’s own serum, 75 mlU/ml FSH + LH (Humecon, Organon, Scarborough, ON, Canada) at 37°C in an atmosphere of 5% CO2 in air for 48 h.

After 24 and 48 h of incubation, mature oocytes were demucleated of cumulus cells using finely drawn glass pipettes following 1 min exposure to 0.1% hyaluronidase solution (Medi-Cult, Hopkinton, MA, USA). Spermatozoa for ICSI were prepared by gradient separation (45 and 90% gradients) at 560 g for 20 min. Following gradient separation, the sperm pellet was washed twice (200 g) with 5 ml of IVF medium (Medi-Cult Inc.). After ICSI, each oocyte was transferred to 20 μl of Medi-Cult medium under mineral oil (Sigma) for culture. Fertilization was assessed 16–18 h after ICSI for the appearance of two distinct pronuclei (2PN) and two polar bodies. Fertilized 2PN oocytes were transferred to 1.0 ml of Medi-Cult medium in two-well organ tissue culture dishes for a further 24 h. For the preparation of the endometrium, the patient was given 4 mg of oestradiol (Estrace; Roberts Pharmaceutical, Mississauga, Canada) in divided doses starting on the day of oocyte retrieval. Luteal support was provided in the form of 200 mg progesterone (Prometrium) twice a day for 16 days starting 48 h after oocyte collection.

A total of 63 oocytes were retrieved (10 with abnormal morphology, 22 at metaphase-I stage and 31 at germinal vesicle stage). Following maturation in culture for 24 h, 22 oocytes were noted to be at metaphase-II stage. After ICSI of the 22 oocytes, 15 were observed to have 2PN on the second day. Of 15 fertilized oocytes 13 cleaved. A total of 3 embryos (one 3-cell stage grade 1, two 4-cell stage grade 3) were transferred at 48 h following ICSI. At embryo transfer, the endometrial thickness was 8.9 mm at transvaginal ultrasound scan. Embryo transfer was performed using a Wallace catheter (SIMS Protx Limited, Hythe, Kent, UK), and two weeks later the serum β-HCG level was 122.2 IU/l. Six weeks after embryo transfer an ongoing intrauterine singleton pregnancy with fetal heartbeat was confirmed by transvaginal ultrasonography. Unfortunately, this pregnancy miscarried at eight weeks after embryo transfer.

Following maturation in culture for 48 h of the remaining 31 oocytes, 19 oocytes were at metaphase-II stage. After ICSI of those oocytes, 16 oocytes were observed to have 2PN. These 16 fertilized oocytes were cryopreserved immediately after fertilization assessment. Embryo freezing was performed by the slow freezing protocol (Lassalle et al., 1985). Briefly, 2PN oocytes were suspended for 20 min in 2 ml of cryoprotectant (1.5 mol/l propanediol, 0.1 mol/l sucrose) in Dulbecco’s phosphate-buffered saline (PBS; GIBCO BRL, Rockville, MD, USA) containing 30% human serum albumin solution (5% human albumin; Bayer, Elkhart, IN, USA). They were then aspirated into 0.25 ml straws (CryoBioSystem, Orsay, France) in a small central droplet of cryoprotectant surrounded by two air bubbles and adjacent cryoprotectant droplets. The straws were then frozen horizontally in a programmable freezer (Planer Kryo 10 Series III; Planer Products Ltd., Sunbury-on-Thames, UK) using a slow-freezing protocol (–2°C/min to –7°C; hold at –7°C for 10 min and manual seeding; –0.3°C/min to –30°C; –50°C/min to –180°C, hold at –180°C for 20 min and then plunge into liquid nitrogen). Thawing was performed by maintaining the straws first in air for 10–15 sec and then plunging them into a water bath at 30°C for 30 sec. The central droplet, containing the 2PN oocytes was then gently expelled from the straw in 2 ml of PBS containing 1 mol/l propanediol (PROH) and 0.2 mol/l sucrose. The 2PN oocytes were then washed free of the cryoprotectants by sequential incubation (10 min) in three aliquots of PBS solution (2 ml) containing lowered molar concentrations of PROH/sucrose (0.5/0.2; 0/0.2; 0/0).

Standard protocol was followed for the preparation of the endometrium for frozen embryo transfer. Briefly, the patient received intravaginal progesterone (Prometrium) in a dose of 300 mg a night for 10 days to induce a withdrawal bleed. After stopping the progesterone, menstrual bleeding started in 3 days. On day 2 of the cycle, the patient was given 200 μg of the gonadotrophin-releasing hormone agonist, Buserelin (Suprefact Synthetic LH-RH analogue; Hoechst Marion Roussel Canada Inc., Laval, Quebec, Canada) and 6 mg oestradiol (Estrace) daily for 10 days. Two days before embryo transfer, Buserelin was stopped and progesterone was administered vaginally (200 mg twice a day).

Three months after cryopreservation, nine fertilized oocytes were thawed for scheduled embryo transfer. Following thawing, 8 fertilized oocytes survived (88.9%; 8/9) and 7 cleaved (87.5%). Three embryos were transferred (one 4-cell stage grade 2, two 4-cell stage grade 3). The endometrial thickness measured 9.0 mm on the day of embryo transfer. However, after two weeks no pregnancy ensued as confirmed by a negative pregnancy test. The second frozen embryo transfer
was performed 5 months later. The remaining 7 fertilized oocytes were thawed and all survived (100%; 7/7). After 24 h of culture, all embryos had cleaved (100%; 7/7). Three embryos were transferred (two 4-cell stage grade 2 and one 4-cell stage grade 3). The endometrial thickness measured 9.5 mm on the day of embryo transfer. Two weeks later, the serum β-HCG concentration was 258.5 IU/l and 6 weeks after embryo transfer an ongoing intrauterine singleton pregnancy with fetal heartbeat was confirmed by transvaginal ultrasonography. An uneventful delivery at 38.5 weeks resulted in the birth of a normal healthy boy (3,040 g).

**Discussion**

Our results demonstrate that the embryos produced by in-vitro matured oocytes retrieved from women with PCOS can be cryopreserved and result in the delivery of a healthy infant following embryo transfer. PCOS is the most common reproductive disorder in women of childbearing age. It is associated with a heterogeneous group of symptoms, including hyperandrogenism and anovulation (Goldzieher and Axelrod, 1963; Yen, 1980). The exact mechanism by which anovulation occurs in PCOS is unknown. There is consensus that the common feature in women with PCOS is arrested follicular development at the stage when selection of the dominant follicle should normally occur (Erickson and Yen, 1984). The initial steps of folliculogenesis, recruitment, and growth to the small antral stage, are functional in PCOS, but the terminal step, selection of dominant follicles that are capable of ovulation, does not occur regularly (Jakimiuk et al., 1997). It is well known that in patients with PCOS, the cyclical pattern of FSH and LH secretion is typically absent, and there is a disproportionately high secretion of LH with a relatively constant low rate of FSH secretion.

The first report of pregnancy in a woman with anovulatory infertility following IVM of immature oocytes and IVF was made in 1994 (Trounson et al., 1994). Recently, it has been demonstrated that recovery of immature oocytes followed by in-vitro maturation of these immature oocytes is a promising treatment for women with PCOS (Chian et al., 1999, 2000). To date, approximately 25–30% clinical pregnancy rates were established from immature oocytes retrieved from patients with PCOS following IVM in several infertility centres (Cha et al., 2000; personal communication). Therefore, these data suggest that women with PCOS-related infertility could be treated with immature oocyte retrieval followed by in-vitro maturation and fertilization, as well as cryopreservation.

Embryos survive and implant at higher rates when frozen at the pronuclear stage compared with the cleavage stage (Quinn, 1990; Demoulin et al., 1991; Veeck et al., 1993). Recent reports also show that embryo cryopreservation at the pronuclear stage optimizes the chance for a live born infant from a single oocyte retrieval (Damario et al., 2000; Senn et al., 2000). Our results show that embryo survival rates were high following freeze–thawing at the 2PN stage (88.9% and 100.0%). Our previous results also show that survival rates of embryos produced by in-vitro matured oocytes are higher when frozen at the 2PN stage compared with the cleavage stage (unpublished data). In conclusion, the embryos produced from in-vitro matured oocytes retrieved from untreated women with PCOS can be safely cryopreserved and healthy infants can be delivered following embryo transfer.

**References**


Senn, A., Vozzi, C., Chanson, A. et al. (2000) Prospective randomized study of two cryopreservation policies avoiding embryo selection: the pronucleate maturation and fertilization, as well as cryopreservation.


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